

APPENDIX A

1. (once amended) A method for detecting in a sample a non-viral organism belonging to a group, the group consisting of at least one, but less than all non-viral organisms, the method comprising the steps of:

(i) contacting a sample comprising SRP RNA with a nucleic acid probe, wherein the nucleic acid probe is substantially complementary to a subsequence of SRP RNA from the group of non-viral organisms;

(ii) incubating the sample comprising SRP RNA and the nucleic acid probe under hybridization conditions such that the nucleic acid probe hybridizes to SRP RNA from the group of non-viral organisms but does not detectably hybridize to SRP RNA from other non-viral organisms that do not belong to the group; and,

(iii) detecting hybridization of the nucleic acid probe to SRP RNA; wherein hybridization of the probe is indicative of the presence of said non-viral organism in the sample.

2. (as filed) The method of claim 1, wherein the nucleic acid probe comprises a detectable moiety.

3. (as filed) The method of claim 1, wherein the SRP RNA comprises a detectable moiety.

4. (as filed) The method of claim 1, wherein the step of contacting further comprises the use of one or more additional nucleic acid probes that are substantially complementary to a subsequence of SRP RNA from the non-viral organism and have the ability to hybridize under stringent conditions to the SRP RNA from the non-viral organism.

5. (as filed) The method of claim 4, wherein one of the nucleic acid probes comprises a detectable moiety.
6. (as filed) The method of claim 1, wherein the nucleic acid probe is about 8 to about 50 nucleotides in length.
7. (as filed) The method of claim 1, wherein the nucleic acid probe is about 15 to about 25 nucleotides in length.
8. (as filed) The method of claim 1, wherein the nucleic acid probe is selected from the group consisting of DNA, PNA, and 2'-O-methyl RNA.
9. (as filed) The method of claim 1, wherein the nucleic acid probe is a PNA.
10. (as filed) The method of claim 1, wherein the nucleic acid probe is perfectly complementary to the subsequence of SRP RNA.
11. (as filed) The method of claim 1, wherein the SRP RNA is 4.5S RNA.
12. (as filed) The method of claim 1, wherein the sample is from a human.
13. (as filed) The method of claim 1, wherein the non-viral organism belonging to the group is a fungus.
14. (as filed) The method of claim 13, wherein the fungus is selected from the group consisting of *Candida* sp., *Cryptococcus* sp., *Aspergillus* sp., *Histoplasma* sp., and *Microsporium* sp.

15. (as filed) The method of claim 1, wherein the non-viral organism belonging to the group is a protozoan.

16. (as filed) The method of claim 15, wherein the protozoan is selected from the group consisting of *Pneumocystis* sp., *Toxoplasma* sp., *Cryptosporidium* sp., *Giardia* sp., *Leshmania* sp., *Trypanosoma* sp., *Plasmodium* sp., *Acanthamoeba* sp., and *Entamoeba* sp.

17. (as filed) The method of claim 1, wherein the non-viral organism belonging to the group is a bacterium.

18. (as filed) The method of claim 17, wherein the bacterium is selected from the group consisting of *Propionibacterium* sp., *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., *Salmonella* sp., *Legionella* sp., *Pseudomonas* sp., *Haemophilus* sp., *Escherichia* sp., *Mycoplasma* sp., *Micrococcus* sp., *Listeria* sp., *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Clostridia* sp., *Neisseria* sp., *Helicobacter* sp., *Vibrio* sp., *Campylobacter* sp., *Bordetella* sp., *Ureaplasma* sp., *Treponema* sp., *Leptospira* sp., *Borrelia* sp., *Actinomyces* sp., *Nocardia* sp., *Chlamydia* sp., *Rickettsia* sp., *Coxiella* sp., *Ehrlichia* sp., *Rochalimaea* sp., *Brucella* sp., *Yersinia* sp., *Fracisella* sp., and *Pasteurella* sp.

19. (once amended) The method of claim 1, wherein the nucleic acid probe has a nucleotide sequence selected from the group consisting of:

GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4); GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:21); CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23);

CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and CGGAUCUGACACG (SEQ ID NO:26).

20. (once amended) A method for detecting in a sample a non-viral organism belonging to a group, the group consisting of at least one, but less than all of non-viral organisms, the method comprising the steps of:

(i) contacting a sample comprising SRP RNA with a first nucleic acid probe, wherein the first nucleic acid probe is substantially complementary to a subsequence of SRP RNA from the group of non-viral organisms and wherein the first nucleic acid probe has the ability to hybridize under stringent conditions to the SRP RNA from the group of non-viral organisms;

(ii) incubating the sample comprising SRP RNA and the first nucleic acid probe under stringent hybridization conditions to form a duplexed SRP RNA from the group of non-viral organisms;

(iii) contacting a duplexed SRP RNA with a gel-immobilized second nucleic acid probe, wherein the gel-immobilized second nucleic acid probe is substantially complementary to a subsequence of a duplexed SRP RNA from the group of non-viral organisms;

(iv) incubating a duplexed SRP RNA and the gel-immobilized second nucleic acid probe under hybridization conditions such that the gel-immobilized second nucleic acid probe hybridizes to a subsequence of a duplexed SRP RNA from the group of organisms, but does not detectably hybridize to SRP RNA from other non-viral organisms that do not belong to the group; and,

(v) detecting hybridization of the gel-immobilized second probe to a duplexed SRP RNA; wherein hybridization of the probe is indicative of the presence of said non-viral organism in the sample.

21. (once amended) The method of claim 20, wherein step (iv) further comprises electrophoresing the sample comprising duplexed SRP RNA through a gel.

22. (once amended) The method of claim 20, wherein the first nucleic acid probe comprises a detectable moiety.

23. (as filed) The method of claim 20, wherein the SRP RNA comprises a detectable moiety.

24. (once amended) The method of claim 20, wherein [the] step (i) of contacting with a first nucleic acid probe further comprises the use of one or more additional nucleic acid probes.

25. (as filed) The method of claim 24, wherein one of the nucleic acid probes comprises a detectable moiety.

26. (once amended) The method of claim 20, wherein the first nucleic acid probe is an adaptor probe comprising a subsequence that hybridizes under stringent conditions to the gel-immobilized second nucleic acid probe.

27. (once amended) The method of claim 20, wherein the gel-immobilized second nucleic acid probe and the first nucleic acid probe each comprise a subsequence that is substantially complementary to the same SRP RNA subsequence.

28. (once amended) The method of claim 20, wherein the gel-immobilized second nucleic acid probe and the first nucleic acid probe are about 8 to about 50 nucleotides in length.

29. (once amended) The method of claim 20, wherein the first nucleic acid probe is about 15 to about 25 nucleotides in length.

30. (once amended) The method of claim 20, wherein the gel-immobilized second nucleic acid probe and the first nucleic acid probe are selected from the group consisting of DNA, PNA, and 2-O-methyl RNA.

31. (once amended) The method of claim 20, wherein the gel-immobilized second nucleic acid probe and the first nucleic acid probe are PNA.

32. (once amended) The method of claim 20, wherein the gel-immobilized second nucleic acid probe is perfectly complementary to the subsequence of SRP RNA.

33. (as filed) The method of claim 20, wherein the SRP RNA is 4.5S RNA.

34. (as filed) The method of claim 20, wherein the sample is from a human.

35. (as filed) The method of claim 20, wherein the non-viral organism belonging to the group is a fungus.

36. (as filed) The method of claim 35, wherein the fungus is selected from the group consisting of *Candida* sp., *Cryptococcus* sp., *Aspergillus* sp., *Histoplasma* sp., and *Microsporum* sp.

37. (as filed) The method of claim 20, wherein the non-viral organism belonging to the group is a protozoan.

38. (as filed) The method of claim 37, wherein the protozoan is selected from the group consisting of *Pneumocystis* sp., *Toxoplasma* sp., *Cryptosporidium* sp., *Giardia* sp., *Leshmania* sp., *Trypanosoma* sp., *Plasmodium* sp., *Acanthamoeba* sp., and *Entamoeba* sp.

39. (as filed) The method of claim 20, wherein the non-viral organism belonging to the group is a bacterium.

40. (as filed) The method of claim 39, wherein the bacterium is selected from the group consisting of *Propionibacterium* sp., *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., *Salmonella* sp., *Legionella* sp., *Pseudomonas* sp., *Haemophilus* sp., *Escherichia* sp., *Mycoplasma* sp., *Micrococcus* sp., *Listeria* sp., *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Clostridia* sp., *Neisseria* sp., *Helicobacter* sp., *Vibrio* sp., *Campylobacter* sp., *Bordetella* sp., *Ureaplasma* sp., *Treponema* sp., *Leptospira* sp., *Borrelia* sp., *Actinomyces* sp., *Nocardia* sp., *Chlamydia* sp., *Rickettsia* sp., *Coxiella* sp., *Ehrlichia* sp., *Rochalimaea* sp., *Brucella* sp., *Yersinia* sp., *Fracisella* sp., and *Pasteurella* sp.

41. (twice amended) The method of claim 20, wherein the gel-immobilized second nucleic acid probe has the nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4); GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:21); CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23); CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and CGGAUCUGACACG (SEQ ID NO:26).

42. (once amended) The method of claim 20, wherein the first nucleic acid probe has a nucleotide sequence selected from the group consisting of:

GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); and
GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8).

43. (as filed) The method of claim 26, wherein the adaptor has a nucleotide sequence selected from the group consisting of:

GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); and
GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8).

51. (new) The method of claim 20, wherein the first nucleic acid probe and the gel-immobilized second probe are perfectly complementary to the SRP RNA.

52. (new) A method for detecting in a bacterium in a sample, the method comprising the steps of:

(i) contacting a sample comprising SRP RNA with a nucleic acid probe that is perfectly complementary to a subsequence of bacterial SRP RNA, wherein the nucleic acid probe is about 8 to about 50 nucleotides in length;

(ii) incubating the sample comprising SRP RNA and the nucleic acid probe under hybridization conditions such that the nucleic acid probe hybridizes under to bacterial SRP RNA but does not detectably hybridize to SRP RNA from non-bacterial organisms; and,

(iii) detecting hybridization of the nucleic acid probe to SRP RNA; wherein hybridization of the probe is indicative of the presence of said bacterium in the sample.

53. (new) The method of claim 52, wherein the nucleic acid probe is selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2);
GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4);
GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7);
GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8);

GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:21); CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23); CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and CGGAUCUGACACG (SEQ ID NO:26).

54. (new) A method for detecting a bacterium in a sample, the method comprising the steps of:

(i) contacting a sample comprising SRP RNA with a first nucleic acid probe that is perfectly complementary to a subsequence of bacterial SRP RNA, wherein the nucleic acid probe is about 8 to about 50 nucleotides in length;

(ii) incubating the sample comprising SRP RNA and the first nucleic acid probe under stringent hybridization conditions to form a duplexed SRP RNA from the bacterium;

(iii) contacting a duplexed SRP RNA with a gel-immobilized second nucleic acid probe, wherein the gel-immobilized second nucleic acid probe is substantially complementary to a subsequence of a duplexed SRP RNA from the bacterium;

(iv) incubating a duplexed SRP RNA and the gel-immobilized second nucleic acid probe under hybridization conditions such that the gel-immobilized second nucleic acid probe hybridizes to a subsequence of a duplex SRP RNA from the bacterium, but does not detectably hybridize to SRP RNA from other non-bacterial organisms; and,

(v) detecting hybridization of the gel-immobilized second probe to a duplexed SRP RNA; wherein hybridization of the probe is indicative of the presence of said bacterium.

55. The method of claim 54, wherein the nucleic acid probe is selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4); GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7);

GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8);
GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG
(SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:21);
CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23);
CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and
CGGAUCUGACACG (SEQ ID NO:26).

56. (new) A method for detecting in a bacterium in human blood sample, the method comprising the steps of:

(i) contacting a sample comprising SRP RNA with a nucleic acid probe that is perfectly complementary to a subsequence of bacterial SRP RNA, wherein the nucleic acid probe is about 8 to about 50 nucleotides in length;

(ii) incubating the blood sample comprising SRP RNA and the nucleic acid probe under hybridization conditions such that the nucleic acid probe hybridizes to SRP RNA from bacterium but does not detectably hybridize to SRP RNA from other non-bacterial organisms; and,

(iii) detecting hybridization of the nucleic acid probe to SRP RNA; wherein hybridization of the probe is indicative of the presence of said bacterium in the blood sample.

57. The method of claim 56, wherein the blood sample is selected from the group consisting of whole blood, plasma, platelets, packed red blood cells, bone marrow, lymphocytes, and serum.

58. The method of claim 56, wherein the nucleic acid probe is selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2);
GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4);
GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7);

GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8);
GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG
(SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:21);
CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23);
CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and
CGGAUCUGACACG (SEQ ID NO:26).

59. (new) A method for detecting a bacterium in a human blood sample, the method comprising the steps of:

(i) contacting a sample comprising SRP RNA with a first nucleic acid probe that is perfectly complementary to a subsequence of bacterial SRP RNA, wherein the nucleic acid probe is about 8 to about 50 nucleotides in length;

(ii) incubating the blood sample comprising SRP RNA and the first nucleic acid probe under stringent hybridization conditions to form a duplexed SRP RNA from the bacterium;

(iii) contacting a duplexed SRP RNA with a gel-immobilized second nucleic acid probe, wherein the gel-immobilized second nucleic acid probe is substantially complementary to a subsequence of a duplexed SRP RNA from the bacterium;

(iv) incubating a duplexed SRP RNA and the gel-immobilized second nucleic acid probe under hybridization conditions such that the gel-immobilized second nucleic acid probe hybridizes to a subsequence of a duplex SRP RNA from the bacterium, but does not detectably hybridize to SRP RNA from other non-bacterial organisms; and,

(v) detecting hybridization of the gel-immobilized second probe to a duplexed SRP RNA; wherein hybridization of the probe is indicative of the presence of said bacterium in the blood sample.

60. (new) The method of claim 59, wherein the blood sample is selected from the group consisting of whole blood, plasma, platelets, packed red blood cells, bone marrow, lymphocytes, and serum.

61. (new) The method of claim 59, wherein the nucleic acid probe is selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4); GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:21); CGGACCTGACCTG (SEQ ID NO:22); AGGACCUGACAUG (SEQ ID NO:23); CGGACCUGACCAG (SEQ ID NO:24); CGGACCUGACAAG (SEQ ID NO:25); and CGGAUCUGACACG (SEQ ID

62. (new) The method of claim 59, wherein the first nucleic acid probe is an adaptor probe comprising a subsequence that hybridizes under stringent conditions to the gel-immobilized probe.

63. (new) The method of claim 62, wherein the adaptor has a nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); and GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8).